

## **Pathophysiology of Stress in Wild and Managed-Care Bottlenose Dolphins (*Tursiops truncatus*)**

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### **LONG-TERM GOALS**

The overall goal of the proposed research is to characterize the pathophysiology of stress in wild and managed-care bottlenose dolphins and to establish relationships between markers of the stress response in cetaceans and immune function, dependent hormonal endpoints, hematology and serum chemistry parameters, biomarkers of stress, inflammation and metabolism and health status.

### **OBJECTIVES**

- Objective 1 – To characterize multiple stress markers in managed-care bottlenose dolphins.
- Objective 2 – To characterize multiple stress markers in semi-domesticated bottlenose dolphins
- Objective 3 – To characterize multiple stress markers in wild bottlenose dolphins
- Objective 4 – To integrate the information obtained from these three populations of bottlenose dolphins in order to develop a validated model of stress and its pathophysiologic effects on the bottlenose dolphin.

### **APPROACH**

We plan to assess baseline stress biomarkers in the following three populations of Atlantic bottlenose dolphins (*Tursiops truncatus*): 1) managed-care (Group 1), 2) semi-domesticated (Group 2), and 3)

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wild (Group 3). This approach will provide a truly comparative study among bottlenose dolphins that live under a range of different and varying stressors.

To develop further understanding on stress in wild and managed-care dolphins and the association between classic measures of stress and new technologies, a research team comprised of scientists from federal, academic, and managed-care marine mammal facilities are collaborating on this project to develop integrated measures of stress using a comparative study design. Additionally, this project are partnering with Dr. Dorian Houser and collaborators in a joint effort for the purpose of integrating traditional markers of stress with novel markers of stress.

## **WORK COMPLETED**

### **SUBTASK 1 - *Collection of samples from Group 1 (managed-care Georgia Aquarium bottlenose dolphins) to characterize multiple stress markers***

In August and September 2011 samples from 6 dolphins were sampled from Georgia Aquarium and a scheduled collection developed for the remainder of the study collection period. Hematological and immune analyses have been completed and remaining analysis will be conducted in FY12.

### **SUBTASK 2 - *Collection of samples from Group 3 (wild bottlenose dolphins)***

In July 2011 samples were collected during dolphin capture–release health assessments conducted in the Indian River Lagoon, FL (IRL) as part of the Dolphin Health and Risk Assessment (HERA) Project. Additional testing was included for this project along with detailed protocols for the sampling, processing, shipping and storage. All animal capture and sampling protocols for the collection of these samples were conducted under National Marine Fisheries Permit No. 14352 (permit dates from 2009-2014) issued to Dr. Gregory Bossart and approved by the Harbor Branch Oceanographic Institutional Animal Care and Use Committee (IACUC). All samples collected were sent to the appropriate laboratories for analysis (with the exception of samples to Dr. Janz which will require CITES Permit and this application has been submitted to the USDA).

### **SUBTASK 3 - *Catecholamine (epinephrine, norepinephrine and dopamine) analysis shall be determined on samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins). These analyses will be conducted by Dr Tracy Romano.***

Samples for catecholamine analysis were sent to Dr. Romano from collection thus far as indicated in Subtasks 2 and 3.

### **SUBTASK 4 - *Immunological assessments for immunophenotyping (B+T cell lymphocyte subsets, MHCII expression) shall be determined on samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins). These analyses will be conducted by Dr Tracy Romano.***

Samples for immunological analysis were sent overnight to Dr. Romano from collections thus far as indicated in Subtasks 2 and 3.

**SUBTASK 5** - *Immunological assessments for lymphocyte proliferation, natural killer cell activity shall be determined on samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins). These analyses will be conducted jointly by Drs. Peden-Adams and Fair.*

Samples for immunological analysis were sent overnight to Drs. Peden-Adams and Fair from collections thus far as indicated in Subtasks 2 and 3.

**SUBTASK 6** - *Immunological analysis of IgG, CRP, and pathogen ELISAs shall be determined on samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins). These analyses will be conducted by Dr. Charles Rice. Samples for these analyses will be collected from dolphins as described in Table 2.*

Samples for immunological analysis were sent to Dr. Rice from collections thus far as indicated in Subtasks 2 and 3.

**SUBTASK 7** - *The following cytokines shall be determined (IL4, IL10, IL17, CD69, TNF $\alpha$ , IFN $\gamma$ , IFN $\alpha$ , MX1, IL2-R $\alpha$ , FADD) in the collected blood samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins) as outlined in Table 2. These analyses will be conducted by Dr. Jeff*

Samples for immunological analysis were sent overnight to Dr Scott from collections thus far as indicated in Subtasks 2 and 3.

**SUBTASK 8** – *Proteomic analysis of samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins) will be conducted by Dr. David Janz.*

A CITES Permit application was submitted to the USDA in order to ship dolphin samples to Dr. Janz for proteomic analysis. Once this is received the samples collected thus far will be sent for proteomic analysis.

**SUBTASK 9** - *Metabolomic analysis of samples from dolphin groups (Group 1 - managed-care bottlenose dolphins; Group 2 – semi-domesticated bottlenose dolphins; Group 3 – wild bottlenose dolphins) will be conducted by Dr. Al Dove in association with colleagues at Georgia Tech, Atlanta, GA. Samples for these analyses will be collected from dolphins as described in Table 2.*

Samples for metabolomic analysis were sent Dr. Dove from collections thus far as indicated in Subtasks 2 and 3.

**SUBTASK 10** - *Data Management, Quality Assurance and Analysis*

The framework used for the Bottlenose Dolphin Health and Risk Assessment Project (HERA) Project was modified to accommodate additional variables for the data collected in this study. As data is received it is QA/QC and incorporated into the Microsoft Access database system.

## RESULTS

The project was initiated in June 2011 as funding was received by both Dr. Fair (N0001411IP20081) and Bossart (N000141110541). Below are listed several accomplishments and further information is presented under subtasks.

1. Preparation of Memorandum of Agreement between U.S. Department of Commerce (NOAA) and U.S. Navy (ONR) for transfer of funding for above project.
2. Preparation of subaward to Georgia Aquarium and U.S. Navy (ONR) for transfer of funding for above project.
3. Finalization of detailed sample collection protocols for the three populations of Atlantic bottlenose dolphins that are the focus of this study as described above.
4. Sample collection completed from Group 3 wild dolphins (n=27) in the Indian River Lagoon during a 2 week period (June 13-24).
5. Initiation of sample collection in August 2011 from Group 1 managed-care dolphins at Georgia Aquarium and in partnership with collaborative study on Group 2 semi-domesticated dolphins at the U.S. Navy.
6. A CITES permit application was submitted for samples to be shipped to Dr. Janz in Canada for proteomic analysis.
7. Development of the database framework for hematology, serum chemistry, immune, hormone and stress biomarker data that is being collected for this project.
8. Drs. Fair and Reif attended ONR Stress Workshop on July 20, 2011.

This project is on target for meeting the outlined objectives within specified timeframe.

## IMPACT/APPLICATIONS

Well-characterized baseline stress evaluation using classic stress hormones paired with biomarker expression using new technologies will provide needed information on natural variation and inter-relationships in hormones/biomarkers among different matrices and across populations maintained under differing environmental conditions. The assessment of stress variables and response in managed-care animals will have important implications for the assessment and interpretation of stress in wild bottlenose dolphins. Approaches and results developed in this proposal to assess the measurement and burden of stress may also be generalized to other marine mammal species.

In order for the US Navy to understand and assess the physiological condition of animals in the wild, particularly in regions where animals are exposed to acoustic and other anthropogenic stressors, it is important to determine the relationship of stress measures not only between tissue matrices but also between managed-care and wild dolphins. This proposal addresses this critical need and furthermore incorporates the use of new technologies to provide an integrative measure of stress with classic parameters extending our knowledge and application of such measures.

## RELATED PROJECTS

The dolphin Health and Risk Assessment Project has several in-press publications that are related or applicable to studies on stress including:

Schaefer, A.M., Bossart, G.D., Mazzoil, M., Fair, P.A., Reif, J.S. Risk factors for colonization of *E. coli* in Atlantic Bottlenose Dolphins (*Tursiops truncatus*) in the Indian River Lagoon, FL. Journal of Environmental and Public Health.

Bossart GD, Schaefer A, Romano T. Peden-Adams M, McCulloch S, Goldstein J, Rice C, Saliki J, Fair P, Reif JS. Clinicoimmunopathologic findings in Atlantic bottlenose dolphins (*Tursiops truncatus*) with positive morbillivirus titers. Diseases of Aquatic Animals.

Fair, P.A., Montie E., Balthis, L. Reif, J. and G.D. Bossart Influence of biological variables and geographic location on circulating concentrations of thyroid hormones in wild bottlenose dolphins (*Tursiops truncatus*). General Comparative Endocrinology.